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PATENT
Atty. Docket No.: 020829-00100US
Client Ref. No.: P433259 TVG/add

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

CHRISTELLER et al.

Application No.: 09/743,690

Filed: May 11, 2001

For: CHIMERIC POLYPEPTIDES ALLOWING EXPRESSION OF PLANT-NOXIOUS PROTEIN

Examiner: Anne R. Kubelik

Art Unit: 1638

Declaration of John Christeller Under 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, John Tane Christeller, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001, and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true and statements made on information or belief are believed to be true.

2. I hold a PhD (1974) in biochemistry from Michigan State University, an MSc (1969) in biochemistry from Victoria University of Wellington (New Zealand), and a BSc in Chemistry & Biochemistry (1968) from Victoria University of Wellington (New Zealand). I am presently employed as a Senior Scientist by the

Horticulture and Food Research Institute of New Zealand Limited. My duties include Programme Manager for Insect Science, Biological Safety Officer, Principal Radiochemical Officer, and Containment Facilities Manager at the Palmerston North site.

3. My field of expertise is insect and microbial biochemistry and molecular biology and I have worked in this field since 1986. Previously I worked in the field of plant biochemistry and molecular biology since 1974. A list of my publications in these fields is attached as Appendix A. A list of distinctions and honors I have received is attached as Appendix B.

4. I have read and am familiar with the contents of the subject patent application. I have also read the Patent and Trademark Office Actions dated November 19, 2002 and August 21, 2003.

5. This invention is directed to transformed plants expressing an exogenous gene construct such that they become toxic to insect pests. Particularly, the invention is directed to chimeric nucleic acid molecules, vectors comprising a nucleic acid molecule of the invention, cells transformed with a vector of the invention, methods of producing a polypeptide encoded by a nucleic acid molecule of the invention, methods of producing pest resistant plants, pest resistant transgenic plants comprising a nucleic acid molecule of the invention, and seed from such plants. The presently claimed invention provides a nucleic acid molecule encoding a polypeptide comprising a vacuole targeting sequence and a plant-noxious pest control sequence linked in operable combination to said vacuole targeting sequence. The plant-noxious pest control sequence is a biotin binding sequence or a functionally equivalent variant or fragment of the biotin binding sequence. It is the biotin binding sequence that provides a plant expressing the chimeric nucleic acid molecule with its toxicity to insects.

I. One of ordinary skill in the art could readily determine the structural and physical characteristics of vacuole targeting sequences and biotin binding sequences

6. It is my understanding that the Examiner has rejected the previously pending claims as allegedly lacking written description, in particular for the recitation of the terms "vacuole targeting sequence" and "biotin binding sequence."

7. Based on the state of knowledge in the field, the terms "vacuole targeting sequence" and "biotin binding sequence" would be well understood by one of ordinary skill in the art and a skilled worker could readily determine the structural and physical characteristics of vacuole targeting sequences and biotin binding sequences useful within the scope of the presently claimed invention. A skilled worker would understand that the term "vacuole targeting sequence" means an amino acid sequence that is operable to direct or sort a selected non-vacuolar protein to which the targeting sequence is linked, to a plant vacuole, or a nucleic acid sequence encoding such an amino acid sequence. A skilled worker would also understand that the term "biotin binding sequence" means an amino acid sequence that is able to bind biotin, or a nucleic acid sequence encoding such an amino acid sequence.

8. A representative list of previously reported plant and fungal vacuole targeting sequences that would be known to one of ordinary skill in the art is attached as Appendix C. By following the teaching of these previous reports and the disclosures of the present specification (see, e.g., page 11, line 24 to page 12, line 1), a skilled worker could readily identify, obtain or manufacture through known means and employing known techniques a vacuole targeting sequence that would be useful within the scope of presently claimed invention.

9. Furthermore, a skilled worker could readily confirm the suitability of candidate vacuole targeting sequences through known means and employing known techniques. For example, by making a homology comparison to known vacuole targeting sequences or alternatively by conducting an experiment whereby the candidate sequence is included in a chimeric nucleic acid of the invention, transformed into a plant and the methodology of Example 4 (see, e.g., page 31, line 12 to page 32, line 40) employed to

confirm vacuole targeting. Alternatively, a candidate vacuole targeting sequence could be included in a chimeric construct that includes a reporter gene such as green fluorescent protein or β-glucuronidase, transformed into a plant and well established methods for identifying reporter gene expression employed to confirm vacuole targeting (e.g. Gallagher SR (1992), "GUS Protocols: using the GUS gene as a reporter of gene expression", Academic Press, San Diego, California, 221 pp., and references therein; and Hicks BW (2002), "Green Fluorescent protein: Applications and protocols", Humana Press, Totowa, New Jersey, 393 pp., and references therein).

10. A representative list of previously reported biotin binding sequences that would be known to one of ordinary skill in the art is attached as Appendix D. Selected references are expanded upon in Appendix E. By following the teaching of these previous reports and the disclosure of the present specification (see, e.g, page 13, line 19 to page 14, line 19), a skilled worker could readily identify, obtain or manufacture through known means and employing known techniques a biotin binding sequence that would be useful within the scope of the present claims.

11. Furthermore, a skilled worker could readily confirm the suitability of candidate biotin binding sequences through known means and employing known techniques. For example, by making a homology comparison to known biotin binding sequences or alternatively by conducting an experiment whereby the candidate sequence is subjected to an *in vitro* biotin binding assay to determine whether it is capable of binding biotin (e.g. Wilcheck M, Bayer EA (1990), "Avidin-Biotin Technology", Methods in Enzymology, 184: 208-240, and articles and references therein). Alternatively, or in addition, a candidate sequence could be subjected to an *in vitro* competitive binding assay by an ordinarily skilled worker using a previously reported biotin binding sequence (such as avidin or streptavidin) to determine the likely suitability of the candidate for inclusion within a nucleic acid of the invention. Multiple candidate sequences could readily be assessed for suitability by a skilled worker using reported library screening methods (manual or automated) employing such assays.

12. The structural and physical characteristics of a reported, putative or candidate vacuole targeting sequence or biotin binding sequence could be readily determined by an ordinarily skilled worker using reported amino acid or nucleic acid sequencing techniques or application of algorithms (e.g. Emanuelsson O, von Heijne G (2001), "Prediction of organellar targeting signals", Biochim. Biophys. Acta 1541: 114-9). If only the amino acid sequence were known, a suitable nucleic acid sequence could be constructed through known means and tested for suitability as described above. The structural and physical characteristics of multiple sequences could be determined using known manual or automated sequencing methods.

13. In addition, none of the analysis required of an ordinarily skilled worker to identify, obtain and characterize suitable sequences for use within a nucleic acid of the invention would constitute undue experimentation. The means and techniques employed to conduct such an analysis are routine in a laboratory specializing in plant biochemistry and genetics.

14. Therefore, one of ordinary skill in the art would find that the claims have sufficient written description for the terms "vacuole targeting sequence" and "biotin binding sequence".

II. One of ordinary skill in the art would interpret "a functionally equivalent variant or fragment of the biotin binding sequence" to exclude variants or fragments where substitution results in loss of biotin binding function and the documents recited in Appendix D support this fact

15. It is my understanding that the Examiner has rejected the claims as non-enabled and alleges that the specification fails to provide guidance for nucleic acids encoding amino acids that bind biotin, and variants and functional equivalents of such amino acids or nucleic acids.

16. As explained in detail above, any ordinarily skilled worker could readily identify and obtain any sequence that is able to bind biotin and employ same within a nucleic acid of the invention as presently claimed.

17. Furthermore, one of ordinary skill in the art would interpret the language of the present claims that reads "a functionally equivalent variant or fragment of the biotin binding sequence" to exclude variant or fragment amino acid sequences where substitution results in loss of biotin binding function. The teaching of the present specification clearly relates to expression of a chimeric polypeptide that is targeted to a plant vacuole and is able to bind biotin. The presently claimed invention is directed to "a *functionally equivalent* variant or fragment of the biotin binding sequence". On reading the disclosure claims of the present specification, a skilled worker would interpret the function of the biotin binding sequence to be a biotin binding function. Accordingly, the phrase a "functionally equivalent variant or fragment of the biotin binding sequence" would be interpreted by a skilled worker to mean a sequence that is varied in some way from, or is a portion of, a reported or newly discovered biotin binding sequence that is still able to bind biotin. The ability of a variant or fragment to bind biotin can be confirmed by conducting a biotin binding experiment as described above.

18. Furthermore, a number of functionally equivalent variants or fragments of previously reported biotin binding sequences have also been reported. In this regard, a representative selection of the references cited in Appendix D is expanded on in Appendix E.

19. Based on the teaching of the present specification, the state of knowledge in the field, a review of the literature and on an analysis of the references cited in Appendix D and explained in Appendix E, one of ordinary skill in the art would conclude that the specification does provide guidance for nucleic acids that bind biotin, and variants and functional equivalents of such nucleic acids.

III. One of skill in the art would be able to identify any useful biotin-binding sequence or functionally equivalent fragment or variant thereof for use within a chimeric construct of the invention

20. It is my understanding that the Examiner has rejected the claims as non-enabled and alleges that the specification does not provide sufficient guidance for

one of ordinary skill in the art to identify any useful biotin-binding sequence or functionally equivalent fragment or variant thereof for use within a chimeric construct of the invention.

21. As explained in detail above, based on the teaching of the present specification, the state of knowledge in the field, a review of the literature and on an analysis of the references cited in Appendix D and explained in E, one of ordinary skill in the art would be able to identify any useful biotin-binding sequence or functionally equivalent fragment or variant thereof for use within a chimeric construct of the invention.

IV. One of skill in the art would be able to produce and test any plant produced with the claimed constructs without undue experimentation and the teaching of the present specification and the references in Appendix F support this fact

22. It is my understanding that the Examiner has rejected the claims as non-enabled and alleges that the specification does not enable one of skill in the art to produce and test any plant produced with the claimed constructs without undue experimentation.

23. The disclosure of present specification teaches in detail regarding the production of transformed plants of the invention (see, e.g., page 16, line 29 to page 21, line 33). In addition, a skilled worker has access to a body of reported transformation protocols, such as those set out in Appendix F.

24. Furthermore, at least Examples 4 to 6 (see, e.g., page 31, line 12 to page 37, line 27) provide clear teaching on preferred methods of testing plants of the invention for the presence of the expressed biotin binding sequence, correct targeting of the biotin binding sequence to the vacuole and toxicity. It is already well established that biotin binding peptides can be toxic to insects and a simple feeding trial can be run to confirm whether a given biotin binding sequence or variant or fragment thereof is in fact toxic. On that basis, a plant transformed according to the invention need only be tested

using an assay to determine that the biotin biding sequence is being expressed at a suitable level, such as the assay disclosed in Examples 4 and 5.

25. Based on the teaching of the present specification, the state of knowledge in the field and a review of the literature, one of ordinary skill in the art would be able to produce and test any plant produced with the claimed constructs without undue experimentation.

The Declarant has nothing further to say.

Dated: 17/12/2003 By: John T. Christeller
John Christeller, Ph.D.

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APPENDIX A

Publications

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APPENDIX B

1981	Department of Scientific and Industrial Research (DSIR) Study Award
1981-1982	Senior Fellow, Japan Society for Promotion of Science
1989	OECD Fellowship, Sustainable Agricultural Systems
1992-1994	DSIR - Massey University Postdoctoral; Fellow Award
1993	Tripartite STC Award
1996	Co-principal Investigator, Marsden Award
1997	ISAT Fellowship
1997	Chairman: FAO Expert Consultative Meeting, New Dehli
1997	OECD Fellowship, Sustainable Agricultural Systems
1998	Visiting Professor, Dept. Entomology, Univ. Maryland
1999	Honorary Lecturer, Microbiology Dept., Otago University
2000	Principal Investigator, Marsden Award

APPENDIX C

Plant and Fungal Vacuole Targeting Sequences

Source	Type	Reference
Plant		
Proteinase inhibitor I	N-terminal extension	Beuning et al., 1994
Proteinase inhibitor II	N-terminal extension	Murray and Christeller, 1994
Aleurain	N-terminal extension	Holwerda et al., 1992
Patatin	N-terminal extension	Sonnewald et al., 1991
Sweet potato sporamin	N-terminal extension	Matsuoka et al., 1990
Barley lectin	C-terminal extension	Bednarek et al., 1990
2S albumin	C-terminal extension	Saalbach et al., 1996
Wheat germ agglutinin	C-terminal extension	Raikhel and Wilkins, 1987
Rice lectin	C-terminal extension	Wilkins and Raikhel, 1989
Tobacco 1,3-glucanases	C-terminal extension	Bol et al., 1990
Tobacco chitinase A	C-terminal extension	Neuhaus et al., 1991
Bean phaseolin	C-terminal extension	Frigerio et al, 1998
Legumin	Internal sequence determinants, C-terminal extension	Saalbach et al., 1991
Bean phytohemagglutinin	Internal sequence determinants	Tague at al., 1990
Ricin	Internal sequence determinants	Frigerio et al., 2001
Tonoplast intrinsic protein	Targeting receptor	Jauh et al., 1998; Jiang and Sun, 2002
Tobacco BP-80	Targeting receptor	Miller et al., 1999; Jiang and Sun, 2002
Arabidopsis AtELP	Targeting receptor	Ahmed et al., 2000
Yeast		
Yeast calnexin	C-terminal extension	Barrieu and Chrispeels, 1999

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APPENDIX D

Biotin Binding Proteins

Biotin Binding Protein	Type	Reference
Avidin	Chicken avidin	Gope et al., 1987
Avidin functionally equivalent variants and fragments	Avidin-related genes	Ahlroth et al., 2000; Keinanen et al., 1994;
	Other bird avidins	Hytonen et al., 2003; Korpela et al., 1981;
	Reptilian avidins	Korpela et al., 1981;
	Amphibian avidins	Green, 1975;
	Charge variant avidins	Marttila et al., 1998;
	Non-glycosylatable avidin	Marttila et al., 2000;
	Monomeric avidin	Laitenen et al., 2002;
	Dimeric avidin	Pazy et al., 2003.
Yolk/plasma biotin-binding proteins	BBP-I, BBP-II	Subramanian and Ariga, 1995; Seshagiri and Ariga, 1987; White and Whitehead, 1987.
Streptavidin	From *Streptomyces avidinii*	Argarana et al., 1986.
Streptavidin functionally equivalent variants and fragments	From *Streptomyces violaceus*	Bayer et al., 1995; Sano et al., 1995;
	Core streptavidin	Thompson and Weber, 1993;
	Dimeric streptavidin	Pazy et al., 2003.
Biotin-binding antibodies	Monoclonal antibody	Dakshinamurti and Rector, 1990
Antibody functionally equivalent variants and fragments	Single chain antibody	Krebber et al., 1997.
Biotin holocarboxylase synthetase	Biotin-binding enzyme	Eisenberg et al., 1982;
	Plant cDNAs	Tissot et a., 1997.
Biotinidase	Biotin-recycling enzyme	Wolf et al., 1990;
	Human cDNA	Cole et al., 1994.
Biotin carboxyl carrier protein (subunit or domain)*	Biotin-containing enzymes	Moss and Lane, 1971;
	3-MeC-CoA carboxylase	Jitrapakdee and Wallace; 2003; Hoffman et al., 1987.
Seed biotin-binding protein (SBP)	Biotin-binding storage protein	Duval et al., 1994; Hsing et al., 1998.

Notes

* Includes the following: acetyl-CoA carboxylase, pyruvate carboxylase, 3-methylcrotonyl-CoA carboxylase, geranoyl- CoA carboxylase, propionyl-CoA carboxylase (specifically the biotin carboxyl carrier protein subunit or domain of these proteins).

NCBI Genbank lists 43 synthetic streptavidins.

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APPENDIX E

Avidin functionally equivalent variants and fragments

1. Ahlroth et al. (2000) and Keinanen et al. (1994) describe the existence of avidin-related genes (AVR). These AVR genes are 91-95% identical to AVD (chicken avidin) in amino acid sequences, including sequences directly involved in biotin-binding. There is therefore a high level of certainty that these gene products are biotin-binding proteins.

2. Korpela et al. (1981) and Hytonen et al. (2003) describe avidins isolated from the egg white of birds other than chicken. Korpela et al. describe egg white and egg yolk binding proteins from 32 species of birds having properties similar to avidin and to chicken egg yolk-biotin-binding protein respectively. Hytonen et al. describe avidins from duck, goose, ostrich and turkey egg white have properties are very similar to chicken avidin regarding structure, glycosylation, heat and protease stability but show different immunological cross-reactivities. N-terminal sequencing of about one quarter of the molecule showed ostrich to be only 50% identical in amino acids whereas the others were much more similar to chicken. The portion sequenced does not include the binding sites where a higher homology is possible. The work shows that these proteins are biotin-binding proteins.

3. Avidin has been identified in eggs of other oviparous vertebrates, reptilians (Korpela et al., 1981) and amphibians (Green 1975). The work shows that these proteins are biotin-binding proteins.

4. Lab-based variants of avidin which continue to bind biotin have been developed. Technological applications often use streptavidin rather than avidin because of the high pI (10.1) of avidin leading to charge-related non-specific binding and because of the presence of glycosylation. Marttila et al. ((1998) created a series

of charge variants with pIs ranging from 9.4 down to 4.7. These proteins bind biotin in a manner similar to the wild-type avidin and have lowered non-specific binding. These charged variants have been combined with a mutation of Asn17>Ile17 which is longer able to be glycosylated (Marttila et al. (2000). There is active research to create an avidin molecule which still functions as a biotin-binding protein as a dimer or monomer rather than a tetramer (Laitenen et al. (2002; Pazy et al. 2003).

Streptavidin functionally equivalent variants and fragments

1. Streptavidin was isolated from S. avidinii. Bayer et al. ((1995) isolated two very similar molecules (1 aa change and 9 aa changes, all very conservative) from S. venesuelae (now S. violaceus). These variants are also biotin-binding proteins.

2. Streptavidin is synthesized as a 159 aa protein in S. avidinii. On isolation a heterogenous mixture of truncated species are found which form aggregates and have poor solubility compared to the fully truncated species. Many synthetic core streptravidins have been made, all of which retain normal functionality (e.g. Sano et al. 1995, Thompson and Leo 1993). These proteins are known as "core" streptavidins.

3. As noted above (Pazy et a. 2003), streptavidin mutants which behave as dimers rather than tetramers, but retain biotin-binding properties, are known.

Biotin-binding antibodies and antibody functionally equivalent variants and fragments

1. A biotin-binding monoclonal antibody has been described (Dakshinamurti and Rector, 1990). In is well known that such molecules can be developed as operable gene sequences known as single chain antibodies (Krebber et al. 1997), such that it would be straightforward to create a biotin-binding SCAB although such a molecule is yet to be described in the literature, so far as is known.

Miscellaneous biotin-binding proteins

1. Two proteins, BBP-I and BBP-II, have beeen described from chickens that are biotin-binding proteins and are present in both the egg yolk and the plasma/plasma biotin-binding proteins (Subramanian and Ariga, 1995; Seshagiri and Ariga, 1987; White and Whitehead, 1987).

2. Seed biotin-binding proteins (SBP) have been isolated and cloned from pea and soybean (Duval et al., 1994; Hsing et al., 1998). They appear to act as biotin-storage proteins, being degraded and releasing biotin during germination. The biotin is bound covalently.

3. Biotin carboxyl carrier protein (subunit or domain) is a biotin-binding component of the nine known biotinylated carboxylases and related enzymes, acetyl-CoA carboxylase, pyruvate carboxylase, 3-methylcrotonyl-CoA carboxylase, geranoyl-CoA carboxylase, propionyl-CoA carboxylase, oxaloacetate decarboxylase, methylmalonyl-CoA decarboxylase, transcarboxylase and urea amidolyase (Moss and Lane 1971; Jitrapakdee and Wallace 2003). There are numerous examples of cloned genes of these proteins in Genbank and the literature. An early example of a plant sequence is given by Hoffman et al. (1987). The biotin is bound covalently.

4. Biotin holocarboxylase synthetase is a biotin-binding enzyme which catalyses the biotinylation of biotin carboxyl carrier proteins. The protein has been characterized and cloned (Eisenberg et al., 1982; Tissot et al., 1997).

5. Biotinidase is an enzyme that functions to salvage biotin from protease-degraded biotin carboxyl carrier protein. Biotin is released as lysyl-biotin (biocytin) and cleaved by biotinidase. Biotinidase has biotin-binding properties and has been characterized and cloned (Wolf et al., 1990; Cole et al., 1994).

APPENDIX F

Plant Transformation

The following are representative publications disclosing genetic transformation protocols that can be used to genetically transform the following plant species:

Rice (Alam et al., 1999, Plant Cell Rep. 18, 572);

Maize (U. S. Patent Serial Nos. 5, 177, 010 and 5, 981, 840);

Wheat (Ortiz et al., 1996, Plant Cell Rep. 15, 1996, 877);

Tomato (U. S. Patent Serial No. 5, 159, 135);

Potato (Kumar et al., 1996 Plant J. 9: 821);

Cassava (Li et al., 1996 Nat. Biotechnology 14, 736);

Lettuce (Michelmore et al., 1987, Plant Cell Rep. 6, 439);

Tobacco (Horsch et al., 1985, Science 227, 1229);

Cotton (U. S. Patent Serial Nos. 5, 846, 797 and 5, 004, 863);

Grasses (U. S. Patent Nos. 5, 187, 073 and 6. 020, 539);

Peppermint (Niu et al., 1998, Plant Cell Rep. 17, 165);

Citrus plants (Pena et al., 1995, Plant Sci. 104, 183);

Caraway (Krens et al., 1997, Plant Cell Rep, 17, 39);

Banana (U. S. Patent Serial No. 5, 792, 935);

Soybean (U. S. Patent Nos. 5, 416, 011; 5, 569, 834; 5, 824, 877; 5, 563, 04455 and 5, 968, 830);

Pineapple (U. S. Patent Serial No. 5, 952, 543);

Poplar (U. S. Patent No. 4, 795, 855);

Monocots in general (U. S. Patent Nos. 5, 591, 616 and 6, 037, 522);

Brassica (U. S. Patent Nos. 5, 188, 958; 5, 463, 174 and 5, 750, 871); and

Cereals (U. S. Patent No. 6, 074, 877).